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Cross-species sperm-FISH assays for chemical testing and assessing paternal risk for chromosomally abnormal pregnancies

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Abstract

The father, like the mother, can transmit genetic defects to his offspring that are detrimental for normal development and a healthy life. Epidemiological studies have identified associations between several paternal exposures and abnormal reproductive outcomes, but they are inherently complex and expensive, and the risk factors for the paternal contribution to abnormal reproductive outcomes remain poorly understood. A series of sensitive methods have been developed for detecting genetic and chromosomal damage directly in sperm, providing potential bioindicators for paternal risk factors for infertility, spontaneous abortions, as well as aneuploidy syndromes, chromosomal rearrangements, and certain genetic diseases in children. Among these, fluorescence in situ hybridization (FISH) has been adapted for the detection of numerical and structural chromosomal abnormalities in the sperm of an expanding number of species including humans and rodents. Sperm FISH has identified several potential paternal risk factors such as age, drugs, lifestyles, and various environmental/occupational exposures. Here, we summarize the status of these sperm-FISH assays and suggest strategies for prioritizing chemical agents for epidemiological investigations to assess paternal risk for abnormal reproductive outcome.

Introduction

Abnormal reproductive outcomes have major emotional and medical expenses to family and society (Committee on Life Science and National

Research Council 1989;U.S.Congress and Office of Technology Assessment 1986). Every year in the United States about 2 million pregnancies are lost before the twentieth week of gestation, about 7% of newborns have low birth weigh, and 5% of babies are born with some birth defect (U.S. Bureau of the Census 1992). More than half of these birth defects have significant effects on the baby's health or viability.

The causes of reproductive abnormalities are diverse and not well understood (Hook 1985). Chromosome abnormalities are an important factor (Chandley 1991; Wyrobek 1993) and about 1% of newborns have a numerical or chromosomal structural abnormality (Committee on Life Science and National Research Council 2000). About half of all spontaneous abortions and a substantial fraction of developmental and morphological birth defects have been associated with *de novo* chromosomal abnormalities (Hassold 1998). Chromosomal abnormalities may arise during gametogenesis of either parent. They may also arise after fertilization when they are likely to result in varying degrees of mosaicism, depending on how early in development they occur. Molecular analyses of the parental origin of abnormal chromosomes during development or after birth indicate that the relative parental contribution to various types of chromosomal abnormalities varies among different categories of defects. Autosomal trisomy appears to be predominantly maternal in origin (e.g., trisomy 21, 18, 16, 13), while sex chromosomal aneuploidies (e.g., 45,X, 47,XXY, 47,XYY, 47,XXX) have a substantial paternal contribution (Hassold et al. 2001). In addition, most *de novo* germinal point mutations and chromosomal structural

rearrangements in offspring appear to arise during spermatogenesis (Chandley 1991).

The human disease burden caused by parental preconception exposure to germinal mutagens is poorly understood, except for a few examples. Of particular concern are cancer chemotherapeutics given before or during the reproductive years (Wyrobek et al. 2004). Each year more than 20,000 children or young persons of reproductive age in the United States are treated with cancer chemotherapeutic regimes, some of which contain known mutagens (Byrne et al. 1998). As treatments become more effective and more patients regain their fertility after treatment, there is concern that chemotherapy-induced germ-line mutations may increase the risk of abnormal reproductive outcomes for these cancer survivors. Certain environmental, lifestyle and occupational exposures have also been shown to affect sperm quality (Wyrobek et al. 1983) also raising concern for the susceptibility of human germ cells. In addition decades of testing for germ-cell mutagenicity using mouse breeding assays for dominant lethality, heritable translocations and specific locus mutations have shown that, when males are treated with a germinal mutagen and mated with unexposed females, the deleterious effects on reproduction can be profound, including infertility, lethality during development, as well as heritable chromosomal translocations, malformations or cancer among offspring (Shelby 1996; Witt et al. 1996). However, these rodent breeding tests are very expensive requiring thousands of animals, which has limited the numbers of agents tested. Also, they do not

always provide information on the underlying mechanism(s) of action, limiting their usefulness for screening potential germ cell mutagens.

Evidence is growing that the risk of abnormal reproductive outcomes of paternal origin may be influenced by a number of factors, such as abnormal male reproductive physiology, predisposing genetic factors (Hassold et al. 2001), past and present male environmental exposures (Olshan et al. 2003) or random errors during sperm production (Crow 2001). Elucidating the relative contribution of each of these factors from epidemiological studies of affected offspring has been difficult because of the small sample sizes of offspring with specific defects and potential bias caused by prenatal selection against chromosomally abnormal embryos. This has provided an incentive for developing methodologies to detect damage directly in sperm.

Direct analysis of human sperm

Chromosomal analysis of male gametes provides a direct approach for studying the paternal contribution to abnormal reproductive outcomes. However, sperm chromosomes have been extremely difficult to study because sperm chromatin is highly condensed and poorly suited for analysis by conventional cytogenetic banding methods. The human-sperm / hamster-oocyte hybrid technique (Rudak et al. 1978) was one of the first approaches for the direct analysis of human paternal chromosomes. This technique has provided estimates that (a) chromosomal aberrations occur at frequencies of 5-7% in normozoospermic, fertile males (Martin et al. 1986), (b) reproducible and

significant variation occurs in frequencies of chromosomally abnormal sperm among healthy men (Genesca et al. 1990), and (c) men exposed to certain genotoxic agents (i.e., chemotherapy, radiotherapy) exhibited higher frequencies of sperm with chromosomal aberrations compared to controls (Martin et al. 1986; Martin et al. 1989; Brandriff et al. 1994; Robbins 1996). However, this technique is exceedingly difficult, labor-intensive and inefficient, dramatically limiting its utility for testing human male exposure to chemicals and the effects of lifestyle factors.

First developed in the late 80s (Pinkel et al. 1988), FISH has proved to be an efficient approach for labelling DNA of chromosomes in all interphase cells, and has been adapted to sperm over a decade ago (Wyrobek et al. 1990; Holmes et al. 1993; Martin et al. 1994; Robbins et al. 1995). Sperm FISH assays have evolved from a one to a two, three, and four-chromosome assay using multiple DNA probes, each specific for a different chromosome. The effectiveness of the technology has improved with the availability of chromosome-specific DNA probes for every human chromosome (Shi et al. 2001) and with the broadly acknowledged emphasis on the importance of scoring criteria (Robbins et al. 1993). Emphasis has also shifted from using any chromosome for which an effective DNA probe was available to selecting chromosomes with clinical relevance for human aneuploidy syndromes (Frias et al. 2003). For example, Figure 1 illustrates a 4-color FISH method (Van Hummelen et al. 1997) that simultaneously utilized four chromosome-specific DNA probes to quantify the frequencies of sperm that are aneuploid for

chromosomes associated with five major aneuploid syndromes among newborns (i.e., trisomy 21, trisomy 18, trisomy 13, Klinefelter, Turner).

Rodent assays for aneuploid sperm

As multicolour sperm-FISH assays were developed for human sperm, similar methods were developed to detect sperm carrying numerical abnormalities and chromosome structural aberrations in laboratory animals. The mouse testicular sperm aneuploidy (mTSA) assay was developed to detect aneuploidy for chromosomes X, Y and 8 in testicular sperm (Wyrobek et al. 1995). The mTSA assay was used to demonstrate increased sperm aneuploidy with advanced male age (Lowe et al. 1995), and elevated frequencies of aneuploid sperm in specific Robertsonion translocation carriers (Baulch et al. 1996). Subsequently, this approach was extended to the analysis of the more homogeneous pool of epididymal sperm, i.e., the mouse epididymal sperm aneuploidy (mESA) assay (Lowe et al. 1996). Aneuploidy data obtained with the mESA method were consistent with those of previous testicular FISH methods and with the literature data for conventional germ-cell meiotic cytogenetic analysis. The spontaneous frequencies of aneuploid sperm in young adult mice and technical factors for the assay were described by Adler et al. (1996) and Schmid et al. (2001). These include harmonizing scoring criteria, rigorous blinding of scorers using procedures that prevent the identification of treated and control animals based on sperm concentration, statistical evaluation of dispersion

characteristics of data in control and treatment groups, and replicating findings in repeated experiments using harmonized scorers.

A multicolor FISH assay was also developed to detect aneuploidy and diploidy in rat sperm using DNA probes for chromosome 4 and Y (Lowe et al. 1998). A probe for a second autosome (chromosome 19) was subsequently added by de Stoppelaar and collaborators (de Stoppelaar et al. 1999), and efforts for developing a 3-chromosome FISH assay utilizing both sex chromosomes to mirror the assays already available for human sperm are underway.

Human and mouse sperm FISH assays for chromosomal structural and numerical abrnormalities

Building on a strategy first described by Eastmond et al. (1994), a human FISH method was developed to detect both numerical and structural abnormalities in human sperm (Van Hummelen et al. 1996). This early sperm FISH method used probes specific for the centromeric and telomeric regions of chromosome 1 and a centromeric probe for chromosome 8 to control for ploidy. It made it possible to simultaneously detect terminal duplications and deletions in chromosome 1p, aneuploidy and diploidy. The assay was validated by comparing the frequencies of chromosomal structural abnormalities with the hamster-egg technology in a reciprocal translocation carrier (Van Hummelen et al. 1997). More recently, an improved sperm-FISH assay (ACM assay, Figure 1b) was developed to detect a broader range of chromosomal structural aberrations in human sperm (Sloter et al. 2000). The ACM assay uses DNA probes specific for

three different regions of chromosome 1 to detect sperm that carry numerical abnormalities or structural aberrations (duplications, deletions and breaks). Baseline frequencies were estimated for sperm with chromosomal aberrations involving chromosome 1 (Table 1) and were consistent with frequencies determined by the hamster-oocyte technique. The ACM method for detecting chromosomal breaks and rearrangements in sperm provides a direct approach for measuring exposure to chromosome-breaking agents and assessing genetic predisposition to such damage.

A FISH assay to detect numerical as well as structural chromosomal abnormalities was recently developed for mouse sperm (Hill et al. 2003)(Figure 3c). This three-color FISH assay (CT8 assay) uses two DNA probes specific for the centromeric and telomeric regions of chromosome 2 plus a probe for the subcentromeric region of chromosome 8. The CT8 assay can detect sperm carrying several types of structural and numerical chromosomal defects such as duplications and deletions of the centromeric or telomeric regions of chromosome 2, disomy 2, disomy 8 and diploidy. The CT8 assay was validated by comparing the frequencies of chromosomal abnormalities in sperm of T(2;14) translocation carriers detected by the CT8 assay against those detected by the cytogenetic analysis of meiosis II spermatocytes using chromosome painting (Hill et al. 2003). The CT8 assay provides the first robust rodent screen for male germ-cell aneugens and clastogens, which includes agents that may lead to increased risks for chromosomally-based developmental defects. An assay for detecting chromosomal structural aberrations is not yet available for rat sperm.

Variation in general population in the frequencies of sperm with chromosomal aberrations

Understanding the sources of variation in the unexposed general population is critical for identifying individuals exposed to potential germ-cell mutagens. Sperm-FISH has been applied to investigate baseline frequencies of defective sperm, attributable to the effects of male age. Age-related increases in sperm carrying disomy Y and X were found in 14 donors aged 22-59 years (Robbins et al. 1995), but no age effect was found for disomy 8. Another study failed to identify an age effect in disomy frequencies of chromosomes 1 and 7 from 18 donors aged 21-49 (Lahdetie et al. 1997). However, an age-effect for disomy 1 and Y was observed in men aged 21-52 years (Martin et al. 1995). In another study, using the human-sperm / hamster-oocyte hybrid technique, the frequency of numerical and structural aberrations was higher in chromosomes of older men, mainly a result of increased nondisjunction, acentric fragments, and complex radial figures (Sartorelli et al. 2001). Overall, the effect of age on chromosomally abnormal sperm remains ambiguous, but overall is much less pronounced in men than it is in women.

Examining geographic and ethnic effects Shi et al. (2000) found no differences in sperm aneuploidy between 10 nonsmoking, non-drinking Chinese men versus Canadians. However, geographic or dietary differences were suggested as possible reasons for differences in frequencies of XY- and disomy-

X-carrying sperm between nonsmokers from the Czech Republic versus California (Rubes et al. 1998).

Recent findings show that men may vary significantly in their baseline frequencies for specific classes of chromosomally abnormal sperm, that variations can persist over years, and that there may be associations between aneuploidy frequencies in sperm and blood (Rubes et al. 2002). The Rubes study also raises concerns about the long-term health consequences of persistently elevated levels of somatic and germ cell aneuploidy. Clearly, additional studies with larger numbers of donors are needed to fully characterize the sources of variations in baseline levels of chromosomally abnormal sperm among human populations and how these variations may affect statistical power to identify germ-cell mutagens.

Status of chemical testing with human and rodent sperm-FISH assays

The literature provides evidence that more than 50 chemicals or chemical mixtures including environmental, occupational and medical exposures can alter semen quality and possibly increase the risk for abnormal reproductive outcomes (Wyrobek et al. 1983; Wyrobek 1993). In the last 10 years, sperm FISH assays have been increasingly employed to identify exposures that increase the frequencies of sperm with chromosomal abnormalities. The following is a synopsis of the current human and rodent data.

Human sperm FISH studies

Table 1 summarizes the studies that utilized human sperm FISH to investigate the effects of lifestyle (Table 1.1), medical (Table 1.2) and occupational (Table 1.3) exposure on the frequencies of sperm with chromosomal abnormalities.

The evidence that smoking, caffeine and alcohol consumption elevates the frequencies of sperm with chromosomal aberrations (Table 1.1) is not consistent. Robbins et al. (1997) found an effect of caffeine on disomy X and XY aneuploidy in sperm but not disomy 18 or Y. They also found a significant association between alcohol intake and disomy X, but no association between smoking and sperm disomy. An effect of cigarette smoking and alcohol consumption lifestyle on sperm aneuploidy was reported by Rubes et al. (1998) but not by Shi et al. (2001). In light of these conflicting results, carefully designed studies with larger numbers of donors will be needed to establish the relationships between sperm aneuploidy and common lifestyle factors.

Several medical exposures (Table 1.2) including chemotherapy regimens have been shown to increase the incidences of chromosomal aneuploidies and diploidies in sperm of treated patients (Martin et al. 1997; Robbins et al. 1997; Martin et al. 1999; De Mas et al. 2001; Frias et al. 2003; see review by Wyrobek et al. 2004). Although the information is limited to a small number of chemotherapies, the increases in aneuploid sperm seem to diminish in semen with increasing time after exposure, suggesting that cancer patients may have only a transient risk for producing higher frequencies of aneuploid offspring after

chemotherapy. A separate drug study, applied a multicolor sperm-FISH assay utilizing probes for chromosome X, Y, and 13 detected significantly elevated levels of sperm disomies in two men who had chronically ingested 0.3 mg/kg/d diazepam for more than 6 months (Baumgartner et al. 2001).

Occupational exposure studies are summarized in Table 1.3. Recio et al. (2001) investigated the effect of exposure to organophosphorous pesticides in men before and during the pesticide spraying season. They reported a significant association between organophosporous metabolite concentration in blood and increased frequencies of sperm aneuploidies. A study of sperm aneuploidy among Chinese pesticide factory workers using the X-Y-18 sperm-FISH assay reported an increase only in disomy Y (Padungtod et al. 1999). A separate study of the effect of pesticides on seasonal outdoor workers using the X-Y-13-21 sperm FISH found no differences in sperm aneuploidy or diploidy between exposed and control groups (Smith et al. 2004). However, the overall exposure levels were smaller in the latter study than those of the study of Padungtod et al. (1999) in which men were in constant contact with pesticides.

Other occupational exposures that have been reported to increase the frequencies of sperm with chromosomal aneuploidies and diploidies in sperm of exposed men include: acrylonitrile which induced sex chromosome non-disjunction (Xu et al. 2003), and benzene which induced aneuploidy and diploidy in sperm of men exposed at concentrations above 10 ppm (Li et al. 2001; Zhao et al. 2004). Recently, benzene exposure has also been shown to increase the frequencies of sperm with chromosomal structural aberrations (Liu et al. 2003).

Finally, occupational exposure to styrene did not have an effect on numerical chromosome aberrations in sperm (Naccarati et al. 2003).

Mouse sperm-FISH assays

To date 12 chemicals have been tested with the mouse sperm-FISH assay for sperm aneuploidy and diploidy (Table 2), including four chemotherapeutics. Taxol was tested at the maximum tolerated dose and the increase of disomic sperm was at the borderline of statistical significance (Adler et al. 2002). Vinblastine gave inconclusive results in repeated experiments in an inter-laboratory comparison (Schmid et al. 1999). Etoposide and merbarone, both topo II inhibitors, showed significant increases in the frequencies of diploid and hyperhaploid sperm (Attia et al. 2002).

Five pharmaceuticals have also been tested by mouse sperm FISH. Diazepam, a tranquilizer, significantly increased the frequency of disomic and diploid sperm in a dose-related manner (Schmid et al. 1999). Griseofulvin, an antifungal drug, increased the frequencies of diploid sperm with the dose, while the frequencies of disomic sperm were increased significantly above the controls in every dose groups but with no clear dose-dependence (Shi et al. 1999). Thiabendazole, a antihelmintic drug, induced only diploid sperm, which suggested that it suppressed the first or second meiotic division (Schmid et al. 1999). The anti-ulcer drug, omeprazol, gave negative results (Adler et al. 2002)

Among the pesticides tested with the mouse sperm FISH assay, carbendazim induced diploidy but not aneuploidy (Adler et al. 2002), while

trichlorfone induced a dose-dependent increase of disomy, but not diploidy (Sun et al. 2000).

Acrylamide, an important industrial chemical used mainly in sewage and waste water treatment plants, showed negative results by sperm-FISH (Schmid et al. 1999). Two doses were tested, the higher one being the maximum tolerated dose. Colchicine, used as positive control, showed increased levels of disomic sperm, but only in the higher concentration (Schmid et al. 1999).

Rat sperm FISH assay

Only two chemicals have been tested by rat sperm FISH (Table 3). The pesticide, carbendazim, was evaluated in epididymal sperm corresponding to exposure during late pachytene of meiosis I. The results showed a clear induction of diploid sperm with a highly significant dose-response relationship but aneuploid sperm were not induced (de Stoppelaar et al. 1999). Chronic low dose treatment of cyclophosphamide (6 mg/kg daily for 9 weeks) of male rats significantly increased the frequency of spermatozoa with chromosome 4 disomy and nullisomy (Barton et al. 2003), but neither disomy Y nor diploidy were significantly increased.

Interspecies comparisons of the effects of chemicals on male germ cells

As the number of sperm FISH assays for investigating the effects of chemical exposure on sperm grows, it is becoming increasingly possible to compare the response among species. A comparison of human and mouse data

suggested that human spermatocytes were 10-100 fold more sensititve to diazepam than those of mice (Baumgartner et al. 2001). These findings, if confirmed, refute the assumption of equal sensitivity of germ cells of both species.

Advantages, statistical power and challenges of sperm FISH tests

The use of FISH methods to detect numerical abnormalities in sperm of human and mammals is gaining in popularity when compared with epidemiological surveys of human offspring or animal breeding studies. It provides the possibility of analyzing large numbers of cells in a relatively short amount of time, provides a high level of sensitivity and statistical power, and small increases can be detected by analyzing sperm from relatively few donors. Table 4 shows the number of sperm samples that would be necessary to detect an increase or decrease of 50% or 100% with the ACM assay (Sloter et al. 2000). The size of the donor groups is dependent on the standard deviation among men in the normal population and the magnitude of the expected effects. Table 4 shows that it is possible to detect a doubling of the frequencies of chromosomal breaks with a sample size of about 10 exposed men and a doubling of the frequency of disomic sperm with a sample size of about 6 exposed men (with an equal number of unexposed men).

There are three major challenges for the sperm-FISH assays that limit its general utility. First, only few chromosomes are investigated in any one assay and it is possible that aberrations rates may not be the same for all the

chromosomes. To minimize this shortcoming, FISH assays are now using probes for up to 4 chromosomes simultaneously, each marked in a different fluorescent color. Additional, the analysis of the same samples using different combinations of probes can further increase the proportion of the genome investigated. Second, the scoring criteria remain subjective and control of technical factors remain critical to the reliability of the sperm-FISH assay, especially when small changes are observed between exposed and control groups. Interlaboratory comparisons have demonstrated the importance of (a) harmonizing scoring criteria, (b) rigorously blind scorers, (c) normalizing cell numbers, (d) evaluating dispersion characteristics of the control and treatment groups and (e) replicating findings in repeated experiments. Third, visual microscopic scoring remain laborious and time-consuming. The development of reliable automated methods (e.g., flow-cytometric analysis or computer-controlled microscopy) are urgently needed to improve the utility of these assays.

Strategies for using the sperm FISH assays for chemical testing and risk assessment

The growing evidence that environmental and occupational exposures can induce increases in the frequencies of sperm with chromosomal abnormalities raises the possibility that we may learn which naturally occurring or man-made chemicals have the potential of inducing chromosomal damage in human germ cells. Because of the time and money necessary to conduct epidemiological studies, it would be cost effective if we were able to prioritize chemical exposures

that have a high potential of constituting a genetic hazard. The availability of sperm FISH assays in a variety of species offers the possibility of implementing a multi-species approach that integrates rodent FISH assays and human somatic cell data for prioritizing chemicals for epidemiology testing for assessing the paternal risk for abnormal reproductive outcome.

Figure 3 illustrates several strategies that employ multi-species sperm FISH to prioritize chemical exposure for epidemiological evaluation. These strategies have the common requirement that chemicals produce positive results in human sperm FISH assays of exposed people (or where human samples are not available, in rodent sperm FISH) before qualifying as candidates for human epidemiological studies. Depending on the toxicology data available, chemical exposures can be assigned into at least 4 priority rank groups:

1. Chemical exposures that induce chromosomal abnormalities in human sperm by sperm FISH assays would make them a candidate for human epidemiological evaluation of paternally mediated abnormal reproductive outcomes.

2. Chemicals that show an effect in the rodent sperm FISH assays would become candidates for testing with the human sperm FISH assays to confirm human sensitivity and to identify exposure parameters before proceeding with time-intensive and expensive human epidemiological studies. A negative finding in the human sperm FISH assay would reduce the priority for studies in exposed human populations.

3. Chemicals for which there is no data in human or rodent germ cells, but have produced positive mutagenicity or clastogenicity results in human somatic cells or rodent somatic cells (e.g., micronucleus) would be candidates for testing with the rodent or human sperm FISH assays, depending on the availability of exposed human subjects. A negative finding by sperm FISH may reduce the priority for further testing.

4. By far the largest group of chemicals have little or no prior testing data for mutagenicity or clastogenicity in any human or animal test system. One may consider prioritizing these by ranking their usage and potential human exposure, and begin testing them by rodent sperm FISH.

This overall strategy offers an integrated approach to concentrate previous epidemiological efforts on chemicals that have a high potential to pose a genetic hazard to humans and those with substantial human exposure. Rodent sperm FISH can be used to identify windows of germ cell sensitivities to refine the experimental parameters to be used in subsequent epidemiological studies.

Conclusions

During the past decade sperm FISH techniques have evolved to provide considerable information about host factors and exogenous exposures that affect the genetic constitution of sperm and the risk for paternally mediated effects on abnormal reproductive outcomes. An integrated interspecies approach to germ

cell genotoxicity takes advantage of the strengths of the human and animal model sperm FISH assays and has the promise of preventing and reducing the incidence of children with paternally transmitted chromosomal defects. Sperm FISH assays may also help us to better understand the mechanisms of germ-cell stage sensitivity for the various types of genetic defects. Of particular concern are persistent effects on stem cells and the effects of chronic exposure on sensitive stages of spermatogenesis. The recently developed sperm FISH assays (human ACM or mouse CT8) for the detection of chromosomal structural aberrations may help for identifying risk factors for chromosomal rearrangements induced in spermatogonia. In addition, studies utilizing mice carrying specific mutations in DNA repair and meiosis genes may help us gain more understanding of the mechanisms of male germ cell genotoxicity and germ-stage sensitivities.

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Table 1.1 Human sperm FISH: Lifestyle factors

Chemical	Chromosomes in FISH assay	No. of exposed	No. of controls	Disomy*			Diploidy*			Reference
Caffeine	18, X, Y	26	19	X Y XY 18	1.2 1.2 1.3 1.4	ns ns 0.05 0.05	X-X-18-18 Y-Y-18-18 X-Y-18-18	1.8 1.8 1.3	0.05 0.05 0.05	Robbins et al., 1997
Alcohol	18, X, Y	32	13	X Y XY 18	1.4 1.6 0.9 1.4	ns ns ns ns	X-X-18-18 Y-Y-18-18 XY1818	2.3 1.7 1.7 1.4	0.05 0.05 0.05	Robbins et al., 1997
Smoking	18, X, Y	17	28	X Y XY 18	1.8 1.5 1.2 1.5	0.05 ns ns 0.05	X-X-18-18 Y-Y-18-18 X-Y-18-18	1.0 0.8 0.7	ns ns ns	Robbins et al., 1997
Smoking	8, X, Y	10	15	X Y XY 8	1.0 2.0 1.4 1.4	ns 0.01 ns 0.01	X-X-8-8 Y-Y-8-8 X-Y-8-8	2.0 1.0 1.2	ns ns ns	Rubes et al., 1998
Smoking	13, X, Y	20	10	X Y XY 13	1.0 1.5 0.8 2.9	Ns Ns Ns 0.01	na	na	na	Shi et al., 2001

* chromosomes, fold increase, p-value

Table 1.2 Human sperm FISH: Effects of medical drugs

Chemical	Chromosomes in FISH assay	No. of exposed	No. of controls	Disomy*			Diploidy*			Reference
NOVP	8, X, Y	4	4	X Y XY 8	2.3 1.7 4.7 3.3	0.05 ns 0.05 0.05	X-X-8-8 Y-Y-8-8 X-Y-8-8	4.3 2.1 2.3	0.05 ns 0.05	Robbins et al., 1997
NOVP	18, 21, X, Y	8	8	X Y XY 18 21	2.2 2.4 12.8 7.1 3.2	0.05 0.05 0.05 0.05 0.05	X-X-18-18-21-21 Y-Y-18-18-21-21 X-Y-18-18-21-21	6.0 4.0 9.0	0.05 0.05 0.05	Frias et al., 2003
BEP	18, X, Y	5	5	X Y XY 18	1.0 4.0 2.6 3.1	ns ns 0.01 0.01	Total Diploid	3.4	0.01	De Mas et al., 2001
BEP	1, 12, X, Y	1	1	X Y XY 1 12	1.2 2.2 2.3 1.5 1.0	ns ns 0.01 ns ns	Total Diploid	1.4	0.01	Martin et al., 1999
BEP	1, 12, X, Y	4	4	X Y XY 1 12	1.0 0.8 0.8 0.6 0.8	ns ns ns ns ns	Total Diploid	1.1	ns	Martin et al., 1997
MACOP-B	1, 12, X, Y	1	5	X Y XY 1 12	0.6 0.5 1.1 1.3 1.5	ns ns ns ns ns	Total Diploid	0.8	ns	Martin et al., 1995
Diazepam	13, X, Y	2	2	X Y XY 13	2.3 1.5 1.5 2.2	0.05 ns ns 0.05	Total Diploid	1.5	ns	Baumgartner et al., 2000

* chromosomes, fold increase, p-value

Table 1.3 Human sperm FISH: Occupational exposure

Chemical	Chromosomes in FISH assay	No. of exposed	No. of controls	Disomy*			Diploidy*			Reference
Pesticides	18, X, Y	9	9	X Y XY 18	1.3 2.4 1.1 1.1	ns ns ns ns	X-X-18-18 Y-Y-18-18 X-Y-18-18	1.9 1.2 1.3	ns ns ns	Recio et al., 2001
Pesticides	13, 21, X, Y	20	20	X Y XY 13 21	0.9 0.9 0.8 0.9 1.2	ns ns ns ns ns	Total Dipoid	0.7	ns	Smith et al., 2004
Pesticides	18, X, Y	13	16	X Y XY 18	1.7 3.5 1.9	ns 0.01 ns	na	na	na	Padungtod et al., 1999
Styrene	2, X, Y	18	13	X Y XY 2	0.7 1.2 0.6 0.8	ns ns ns ns	X-X-2-2 Y-Y-2-2 X-Y-2-2	0.4 0.4 1.8	ns ns ns	Naccarati et al., 2003
Acrylonitrile	X, Y	9	9	X Y XY	1.8 2.3 1.9	0.05 0.05 0.05	na	na	na	Xu et al., 2003
Benzene	7, 8	15	12	7 8	3.2 2.3	0.01 0.01	Total Diploid	2.3	0.01	Zhao et al., 2004
Benzene	9, 18	14	16	9 18	3.4 1.7	0.01 0.01	Total Diploid	1.9	0.01	Li et al., 2001
Benzene	1, 18	15	14	1 18	2.0 2.5	0.01 0.01	Total Diploid	1.0	ns	Liu et al., 2003

* chromosomes, fold increase, p-value

Table 2: Chemicals tested for sperm aneuploidies and diploidies by mouse Sperm-FISH

Chemical	Doses	Aneuploidy	Diploidy	Reference
Taxol	50 mg/kg[1]	-	-	Adler et al., 2002
Vinblastine	0.5 mg/kg[1]	-	-	Schmid et al., 2001a
	1 mg/kg[1]	+/-	-	
	2 mg/kg[1]	+	-	
Etoposide	25 mg/kg[1]	+	+	Attia et al., 2002
	50 mg/kg[1]	+	+	
Merbarone	30 mg/kg[1]	+	+	Attia et al., 2002
	60 mg/kg[1]	+	+	
Diazepam	3 mg/kg[2]	-	-	Adler et al., 2002
Diazepam	75 mg/kg[2]	-	-	Schmid et al., 1999
	150 mg/kg[2]	-	+	
	300 mg/kg[2]	+	+	
Omeprazole	150 mg/kg[1]	-	-	Adler et al., 2002
	300 mg/kg[1]	-	-	
Griseofulvin	500 mg/kg[2]	+	-	Qinghua et al., 1999
	1000 mg/kg[2]	+	+	
	2000 mg/kg[2]	+	+	
Thiabendazole	100 mg/kg[2]	-	-	Schmid et al., 1999
	300 mg/kg[2]	-	+	
Carbendazim	500 mg/kg[2]	-	+	Adler et al., 2002
	1000 mg/kg[2]	-	-	
Trichlorfon	200 mg/kg[1]	+	-	Sun et al., 2000
	300 mg/kg[1]	+	-	
	405 mg/kg[1]	+	-	
Acrylamide	60 mg/kg[1]	-	-	Schmid et al., 1999
	120 mg/kg[1]	-	-	
Colchicine	1.5 mg/kg[1]	-	-	Schmid et al., 1999
	3 mg/kg[1]	+	-	

All these studies used the X-Y-8 sperm-FISH assay

[1] i.p. injection, acute

[2] p.o. application, acute

Table 3: Chemicals tested for sperm aneuploidies and diploidies by rat sperm-FISH

Chemical	Doses	Aneuploidy	Diploidy	Reference
Carbendazim	50 mg/kg[2]	-	-	de Stoppelaar et al., 1999
	150 mg/kg[2]	-	-	
	400 mg/kg[2]	-	+	
	800 mg/kg[2]	-	+	
Cyclophosphamide	6 mg/kg[1]	+	-	Barton et al., 2003

All these studies used the Y-4 sperm-FISH assay

[1] i.p. injection, acute

[2] p.o. application, acute

Table 4: Statistical Power of human sperm-FISH (ACM assay)

		Sample size to detect % increase or decrease[2]	
	Mean[1]	**50%**	**100%**
Segmental aneuploidies			
Duplications and deletions of the 1 pter region	10.8 ± 5.5	22	6
Duplications and deletions of the 1 cen – 1q12 region	1.8 ± 1.6	67	17
Chromosomal breaks			
Breaks between 1 cen and 1q12 and within 1q12	4.6 ± 3.2	41	10
Disomy and Dipoidy			
Disomy 1 or Diploidy	22.7 ± 11.2	21	6

[1] Mean + SD of 10 men (20—30 years, nonsmokers)
[2] Numbers of men in the exposed group with an equal number of controls